DTIC FILE COPY

(2)

SEC

AD-A201 069

MENTATION PAGE

Form Approved
OMB No. 0704-0188

1a (U)	1b. RESTRICTIVE MARKINGS NA
2a. SECURITY CLASSIFICATION AUTHORITY NA	3. DISTRIBUTION/AVAILABILITY OF REPORT Distribution Unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE NA	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) Weizmann Institute of Science	5. MONITORING ORGANIZATION REPORT NUMBER(S) NA
6a. NAME OF PERFORMING ORGANIZATION Weizmann Institute of Science	6b. OFFICE SYMBOL (If applicable) NA
6c. ADDRESS (City, State, and ZIP Code) Weizmann Institute of Science Department of Polymer Research Rehovot 76100, ISRAEL	7a. NAME OF MONITORING ORGANIZATION Office of Naval Research
	7b. ADDRESS (City, State, and ZIP Code) 800 N Quincy St. Arlington, VA 22217-5000 U.S.A.
8a. NAME OF FUNDING/SPONSORING ORGANIZATION Office of Naval Research	8b. OFFICE SYMBOL (If applicable) ONR
8c. ADDRESS (City, State, and ZIP Code) 800 N. Quincy St. Arlington, VA 22217-5000	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER N00014-86-J-1061
	10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
61153N	RR04106	441d005-01	

DTIC ELECTE OCT 19 1988 S E D

11. TITLE (Include Security Classification)
(U) Study of Pure Proteins, Nucleic Acids and their Complexes from Extreme Halobacteria of the Dead Sea: RNA Polymerase-DNA Interaction.

12. PERSONAL AUTHOR(S)
Eisenberg Henryk; Mevarech Moshe (Microbiology, University of Tel-Aviv)

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
ANNUAL	FROM 10/87 TO 10/88	1988/10/10	21

16. SUPPLEMENTARY NOTATION
(ribosomal Ribonucleic acid).

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	
08			Archaebacteria, Halobacteria, Proteins, Nucleic Acids, RNA Polymerase-DNA Interactions, Ribosomal operons, Ribosomal genes. (RAW)

19. ABSTRACT

Two (rRNA) operons of *H. marismortui* were isolated and cloned into the plasmid pBR322 as 10 kb and 20 kb HindIII fragments respectively. A restriction map of the 10 kb clone (pHH10) and of a HindIII-ClaI 8 kb (pHC8) subclone of the other operon were established. Southern hybridization of 16S, 23S and 5S rRNA probes to the clones demonstrates that both operons code for the three rRNA species.

Using S1 analysis, the transcription initiation sites, processing sites of the primary transcripts, as well as the boundaries of the mature rRNA molecules were determined. Comparison of the two operons indicates that they are not identical though both are transcribed *in vivo*. The most striking difference between the operons is the existence of three transcription initiation sites in one operon (HC8) and only one such site in the other one (HH10).

Nucleotide sequence analysis of the 5' flanking sequences of the two operons shows that in the operon containing the three transcription initiation sites there are correspondingly three sequences sharing extensive homology. Only one homologous region was found in the upstream region of the other operon. These putative promoter sites resemble the promoter sites of *H. cutirubrum* . Partial sequence analysis of the two 16S rRNA indicated extensive homology but not identity.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☑ SAME AS RPT. ☐ DTIC USERS	(U)

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
Dr. M. Marron	202/696-4760	ONR

DD Form 1473, JUN 86 — Previous editions are obsolete. — SECURITY CLASSIFICATION OF THIS PAGE

S/N 0102-LF-014-6603

Annual Report

Contract No.: N00014-86-J-1061

Title: Study of Pure Proteins, Nucleic Acids and their Complexes from Extreme Halobacteria of the Dead Sea: RNA Polymerase-DNA Interaction.

P.I. : Henryk Eisenberg
Department of Polymer Research
The Weizmann Institute of Science
Rehovot 76100, Israel

Moshe Mevarech
Department of Microbiology
University of Tel-Aviv
Tel-Aviv 69978, Israel.

Table of Contents

1. Project goals.

The objectives of our program are to isolate and characterize a fully active DNA dependent RNA polymerase from the extremely halophilic archaebacteria from the genus Halobacterium; to isolate and characterize promoter regions of these bacteria; to study the specific polymerase-DNA interactions.

Our goals for the second year of the project were to sequence the two promoter regions of the two rRNA operons of H. marismortui and to analyze homologies in the sequence of the three promoter regions of the HC8 operon and the single promoter region of the HH10 operon. In parallel we planned to develop footprint analysis protocols appropriate for analysis at the extreme salt concentrations extant in the cytoplasm of the halobacteria, to study the interaction of promoter (and transcription processing sites) with the DNA dependent RNA polymerase and its components, present in purified cellular extracts. Ultimately it should then be possible to reconstitute a specifically active RNA polymerase.

2. Accomplishments.

A) Since submitting our first annual report (10/87) we have sequenced and characterized the 5' upstream sequences of the two operons of H. marismortui described in our previous reports. A detailed presentation of our finding, now in process of submittance for publication, follows.

B. Summary

Two rRNA operons of *H. marismortui* were isolated and cloned into the plasmid pBR322 as 10 kb and 20 kb HindIII fragments respectively. A restriction map of the 10 kb clone (pHH10) and of a HindIII-ClaI 8 kb (pHC8) subclone of the other operon were established. Southern hybridization of 16S, 23S and 5S rRNA probes to the clones demonstrates that both operons code for the three rRNA species.

Using SI analysis, the transcription initiation sites, processing sites of the primary transcripts, as well as the boundaries of the mature rRNA molecules were determined. Comparison of the two operons indicates that they are not identical though both are transcribed *in vivo*. The most striking difference between the operons is the existence of three transcription initiation sites in one operon (HC8) and only one such site in the other one (HH10).

Nucleotide sequence analysis of the 5' flanking sequences of the two operons shows that in the operon containing the three transcription initiation sites there are correspondingly three sequences sharing extensive homology. Only one homologous region was found in the upstream region of the other operon. These putative promoter sites resemble the promoter sites of *H. cutirubrum* . Partial sequence analysis of the two 16S rRNA indicated extensive homology but not identity.

C. Introduction

Ribosomes are indispensable components of every living cell and therefore can be used to study evolution on a molecular level. The comparison of the nucleotide sequences of rRNA from different organisms led Woese and his colleagues to suggest that all living organisms can be classified into three phylogenetic kingdoms: the eukaryotes, the eubacteria and the archaebacteria (Woese 1987).

The organization of the rRNA genes varies in the different members of the archaebacteria. In *Thermoplasma acidophilum* the genes coding for 16S , 23S and 5S rRNA are widely separated (Tu and Zillig 1982). In the sulfur metabolizing archaebacteria the distance between the 16S and 23S rRNA genes is variable, ranging between 0.1 kb and 2 kb. The distance between the 23S rRNA gene and the 5S rRNA gene can reach 11 kb as in *Thermoproteus* (Neumann et al 1983). Whereas in the cases described above each genome has only one copy of the 16S and 23S rRNA genes, in the methanogens the number of sets varies, reaching four in *Methanococcus vanielli* (Jarsch et al 1983). More than one copy of 5S rRNA per copies of 16S and 23S rRNA were found in several cases.

The extremely halophilic archaebacteria of the genus *Halobacteriacae* comprise a unique group of bacteria adapted to growth at extreme salinities. *Halobacterium halobium* (Hofman et al 1979) and the closely related *H. cutirubrum* (Hui and Dennis 1985) have only one set of a closely linked 16S-23S-5S rRNA gene cluster. Since the rRNA molecules are structural components of the ribosomes, the growth rate of the organism will depend on the rate of their transcription. The single rRNA gene clusters of these organisms have five, tandemly located, transcriptional initiation site which are believed to increase the efficiency of transcription from these operons (Mankin et al 1984, Hui and Dennis 1985).

Unlike *H. cutirubrum* and *H. halobium* the extremely halophilic *H. marismortui* was found to contain two rRNA operons. The following communication describes the purification and

some properties of these operons.

D. Materials and Methods

Bacterial strains: *H. marismortui* (Ginzburg et al 1970) was obtained from Dr. B.Z. Ginzburg (Hebrew University, Jerusalem). *E. coli* HB101 and 71/18 were used for the recombinant DNA work.

Culture conditions for *Halobacterium* : The description of the culture conditions for *Halobacterium* are described by Mevarech and Werczberger (1985).

Fractionation of Nucleic acids: Bacterial culture (1 liter) was grown for three days to a cell density of $3x10^9$ cells/ml. The cells were harvested in a Sorvall GSA rotor at 10,000 rpm for 10 minutes at 4^0C. The supernatant was removed carefully and the pellet was washed in a solution containing, 204 g NaCl and 39.6 g $MgSO_4$ 7 H_2O, per liter. The washed bacteria were suspended in 100 ml of 10 mM $MgCl_2$, 10 mM Tris HCl pH 7.5 and 100 ml of buffer saturated phenol were added. The mixture was agitated for 30 minutes at 37^0 C and the phases were separated by centrifugation. The aqueous phase was reextracted with phenol, then NaCl was added to a final concentration of 0.5 M and the solution cooled on ice. One hundred ml of cold ethanol was added carefully and the DNA was collected by spooling on a glass rod. The DNA was washed twice with absolute ethanol, twice with ether and air dried. Then it was dissolved in TE (1 mM EDTA, 10 mM Tris HCl pH 7.5) by incubation at 4^0 C. RNA was prepared by adding 100 ml of cold ethanol to the solution left from the DNA preparation and after two hours at -20^0 C, the suspension was centrifuged in a Sorvall SS-34 rotor at 7000 rpm for 10 minutes in the cold. In order to separate the large RNA molecules from the small ones, the pellet was suspended in 1 M NaCl and then left overnight at 4^0 C. The suspension was centrifuged and the large RNA molecules were collected in the pellet. Two and a half volumes of ethanol were added to the supernatant in order to precipitate the 5S rRNA and tRNA species following by cooling to -20^0 C and centrifugation. These RNA species were fractionated by chromatography through a Sephadex G-100 column equilibrated with 1 M NaCl, 10 mM Tris HCl pH 7.5. The large 16S and 23S rRNA molecules were separated by centrifugation through a linear 5-20% sucrose gradient (in 150 mM NaCl, 10 mM Tris HCl pH 7.5) in a Beckman SW56 rotor at 25000 rpm for 18 hours at 4^0 C. Fractions were collected and analyzed by agarose gel electrophoresis.

In vitro labelling of RNA: 16S and 23S rRNA were partially cleaved as follows: 10 µg RNA were dissolved in 50 mM Tris HCl pH 9.5 and transferred to a siliconized 1.5 ml polypropylene test tube. The test tube was tightly closed and incubated at 90^0 C. Incubation time was 20 and 40 minutes for 16S and 23S rRNA respectively. 5S rRNA and tRNA were dephosphorylated by treatment with calf intestinal alkaline phosphatase. Kination was performed at 10 mM $MgCl_2$, 5 mM dithiothreitol, 1 mM spermidine and 50 mM Tris HCl pH 9.5 using 50 µCi γ^{32}P-ATP (3000 Ci/mmole) and 2 units of polynucleotide kinase. The reaction was performed at 37^0 C and terminated after one hour by the addition of 100 µl of 2.5 M ammonium acetate and 2.5 volumes of cold ethanol.

S1 nuclease mapping: Nuclease S1 mapping of rRNA was carried as described by Favaloro et al (1980) using restriction fragments either 3' end labelled with Klenow polymerase and α^{32}P

dNTP or 5' labelled by the phosphate exchange reaction using $\gamma^{32}P$ ATP and polynucleotide kinase. For these experiments RNA was isolated from logarithmically growing cultures (A_{600} of 0.2-0.3).

DNA sequence analysis: Restriction fragments from the cloned genes were subcloned into M13 vector for dideoxy DNA sequencing according to Sanger et al (1980). When necessary, deletions were created using exonuclease III digestions following by mung bean nuclease digestions. All sequences were determined from both strands.

E. Results

Molecular cloning: Genomic DNA was digested by the restriction endonuclease HindIII and then fractionated on a linear sucrose gradient (5-20% in 150 mM NaCl, 2 mM EDTA, 10 mM Tris HCl pH 7.5). The gradient was centrifuged in a Beckman SW40 rotor at 30,000 rpm for 18 hours at 4^0C. Fractions of 0.35 ml were collected. The DNA was precipitated by addition of 0.7 ml of cold ethanol. Aliquots of alternating fractions were analyzed by electrophoresis in a 0.7% agarose gel. The DNA was then transferred to nitrocellulose filters and hybridized to a mixture of 16S and 23S ^{32}P-labeled rRNA. The results are shown in Figure 1. As can be seen the RNA hybridizes strongly to fractions 2 and 10 which contain DNA fragments of 20 kb and 10 kb respectively. The DNA fragments from these fractions were ligated separately to a HindIII digested and alkaline phosphatase treated pBR322 plasmid and the recombinant plasmids were used to transform *E.coli* HB101. Amp^r, Tet^s colonies were transferred to Whatman 540 filter paper and hybridized to ^{32}P-labeled rRNA. Plasmid DNA from positive colonies was extracted and analyzed by digestion with HindIII. Several plasmids containing 10 kb inserts and several plasmids containing 20 kb inserts were further analyzed. Each of the plasmids hybridized to purified 16S and 23S rRNA indicating the existence of two operons of rRNA genes.

A plasmid containing the HindIII-HindIII 10kb insert was denoted pHH10. In order to simplify the analysis of the other operon, the plasmid containing the 20kb insert was trimmed by digestion with ClaI and self ligation resulting in a plasmid containing a ClaI-HindIII 8kb insert. This plasmid was denoted pHC8.

Mapping of the two rRNA operons: Plasmids pHH10 and pHC8 were digested with different restriction enzymes. The restriction maps of nine enzymes were established by a series of digestions and double digestions of the plasmids with different combinations of these enzymes. No site for BamHI was found in these operons. The resulting maps are shown in Figure 2.

Hybridization of 5S rRNA and tRNA: DNA of plasmids pHH10 and pHC8 were digested with various restriction enzymes, electrophoresed and Southern blotted. The blots were hybridized to ^{32}P labelled 5S rRNA or tRNA molecules. A summary of the results is given in Table 1. As can be seen both clusters contain genes for 5S rRNA and tRNA. The positions of the 5S rRNA genes were located 3' to the 23S rRNA by partial sequencing of the 1.8 kb EcoRI-EcoRI fragment of pHC8 and 800 bp EcoRV-EcoRV fragment of pHH10 (data not shown). The exact location of the tRNA genes has not yet been determined .

SI analysis: The 5' and 3' boundaries of the mature 16S and 23S rRNA genes as well as the transcription initiation sites and the processing sites were determined using the method of 'SI

analysis' as described in the 'Methods and materials' section. The results are shown in Figures 3-6. The analyses of the 5' region of the 16S rRNA of the HH10 and HC8 operons were performed using 5' labelled 1.9 kb HindIII-AvaI and 1160 bp AvaI-AvaI fragments respectively. As can be seen (Figure 3) the 5' ends of the mature 16S rRNA molecules (situated 133 bp upstream from the internal AvaI site) are at the same sites. However, a major difference exists between the two operons. Whereas the HH10 operon has only one site for transcription initiation the HC8 operon has three such sites.

Using 3' labelled 630 bp AvaI-AvaI fragments derived from the 3' ends of the two 16S rRNA genes, the positions of the 3' ends were determined to be 108 bp down from the internal AvaI site (Figure 4). The processing sites were, however, different in the two operons.

Using a 5' labelled 670 bp EcoRV-EcoRV fragment of HC8 and a 1650 bp EcoRV-SmaI fragment of HH10 the 5' ends of the mature 23S rRNA genes were determined to be 395 bp from the internal EcoRV site (Figure 5). Two processing sites were detected in the HH10 operon. One is 225 bp and the other 305 bp from the maturation site. This second processing site corresponds to the 310 nucleotide band detected in the 3' SI analysis of the 16S rRNA. Three processing sites were detected in the HC8 operon at positions 165 bp, 205 bp and 225 bp from the maturation site. From these data the space between the 16S and 23S genes in the two operons can be determined to be 510 bp and 870 bp for HH10 and HC8 respectively.

Using a 3' labeled 1.8 kb EcoRI-EcoRI fragment from HH10 and a 2.3 kb fragment from HC8 the 3' ends of the 23S rRNA were determined to be 1250 bp from the internal EcoRI site (Figure 6).

From the data presented one can conclude that although the sizes of the mature rRNA species are the same for the two operons the intragenic spaces as well as the processing sites are different. Moreover, it appears from the information resulting from the SI analyses that both operons are equally transcribed under the conditions used for the preparation of the RNA.

Sequence analysis of the 5' flanking regions of the two operons: Nucleotide sequences were determined starting from the AvaI sites located inside the 16S rRNA genes (133 nucleotides from their 5' maturation sites) and extending about 500 bp into the 5' flanking regions. The nucleotide sequences are given in Figure 7. As expected the two rRNA coding regions are highly homologous, except for 12 out of the 133 nucleotides sequenced. The homology breaks down in the upstream flanking sequences. Near the regions previously determined by the SI analyses to be transcription initiation sites, we again find homologous sequences. In agreement with the SI analyses there are three such regions in the HC8 operon and one in the HH10 operon. Figure 8 summarizes the homologies among the four transcription initiation sites of *H. marismortui* in comparison to those of *H. cutirubrum* (Hui and Dennis, 1985).

F. Discussion

From the hybridization of a radioactively labelled mixture of the different rRNA species to sucrose gradient fractionated HindIII digested genomic *H. marismortui* DNA, we find that in this organism there are two clusters of rRNA genes. These clusters were cloned in pBR322 plasmid and shown to contain a 16S, 23S and 5S rRNA gene set. The organization of the genes in each operon is 5' 16S-23S-5S, as in the other halobacteria. The locations of the boundaries of the 16S and 23S rRNA genes were determined by SI analysis. From these analyses it appears that the sizes of the two 16S and two 23S rRNA genes are the same. However, the sizes of the intergenic spaces are different, being 510 bp in HH10 and 870 bp in HC8.

A comparison of the nucleotide sequence of the first 133 nucleotides of the two 16S rRNA genes reveals significant heterogeneities. There are one insertion of two bases, eight transitions and two transversions in this small region of the gene. Recently, a partial nucleotide sequence of the 16S rRNA of *H. marismortui* was determined by a modified chain termination method in which the rRNA was used as a template and synthetic oligonucleotides were used to prime reverse transcription (Oren et al 1988). In this study the existence of more than one distinct 16S rRNA species was not assumed. In many positions the nucleotides could not be determined unequivocally. This can be understood clearly as several of these positions overlap the mismatches between the two 16S rRNA genes observed by us. If this applies as well to the parts of the genes that have not been sequenced yet, it follows that the two 16S rRNA genes differ significantly. Concerning the 23S rRNA genes, the HC8 gene contains an extra XhoI site which indicates that these two genes are not identical either. Although the genes from the two clusters are not identical, both are transcribed as is seen from the SI analysis. This means that the ribosomes must be heterogeneous as well.

The most striking difference between the two rRNA gene clusters is seen in the 5' flanking regions. SI analysis of these regions reveals three sites for initiation of transcription in the HC8 cluster and one such site in the HH10 cluster. In general the nucleotide sequences of the 5' flanking regions are very different in the two clusters. The only homologous sequences are located close to the previously determined sites of transcription initiation. These highly homologous sequences are homologous also to the putative promoter sequences of the rRNA genes of *H. cutirubrum* and *H. halobium*. In search for common motifs for archaebacterial promoters, it was suggested (Zillig et al 1988) that archaebacterial promoters contain a consensus sequence $^{T}TG^{A}$ near the first nucleotide to be transcribed and a sequence $TTTA^{T}A$ about 20 bases upstream. All the rRNA promoters of halobacteria have these consensus sequences. However, considerable significance must be attached to the fact that much wider regions are conserved in the rRNA operons of these organisms. It is possible that the entire conserved regions are the target of transcription factors which regulate specifically the expression of the rRNA genes. At this stage of our research no attempt was made to quantify the strength of each promoter. It seems however, from the intensity of the SI experiment data, that the single HH10 promoter is as strong as the other three promoters of the HC8 cluster.

G. References

Favaloro, J., Treisma, R. and Kamen, R. (1980) Transcription maps of polyoma virus-specific RNA: analysis by two-dimensional nuclease SI gel mapping. Methods Enzymol. 65: 718-749.

Ginzburg, M., Sachs, L. and Ginzburg, B.Z. (1970) Ion metabolism in *Halobacterium* : I Influence of age on intracellular concentrations. J. Gen. Physiol. 55: 187-207.

Hofman, J.E., Lau, R.H. and Doolittle, W.F. (1979) The number, physical organization and transcription of ribosomal RNA cistrons in an archaebacterium: *Halobacterium halobium* . Nucleic Acids Res. 7: 1321-1333.

Hui, I. and Dennis, P.P. (1985) Characterization of the ribosomal RNA gene clusters in *Halobacterium cutirubrum* . J. Biol. Chem. 260: 899-906.

Jarsch, M., Alterbuchner, J. and Boeck, A. (1983) Physical organization of the genes for

ribosomal RNA in *Methanococcus vannielii.* Mol. Gen. Genet. 189: 41-47.

Mankin, A.S., Teterina, N.L., Rubtsov, P.M., Baratova, L.A. and Kagramanova, V.K. (1984) Putative promoter region of rRNA operon from archaebacterium *Halobacterium halobium*. Nucleic Acids Res. 12: 6537-6546.

Mevarech, M. and Werczberger, R. (1985) Genetic transfer in *Halobacterium volcanii*. J. Bacteriol. 162: 461-462.

Neumann,H.,Gierl, A., Tu, J., Leibrock,J., Staiger, D. and Zillig, W. (1983) Organization of the genes for ribosomal RNA in archaebacteria. Mol. Gen. Genet. 192: 66-72.

Oren, A., Lau, P.P. and Fox, G.E. (1988) The taxonomic status of "*Halobacterium marismortui* " from the Dead Sea: a comparison with *Halobacterium vallismortis*. System. Appl. Microbiol. in press

Sanger, F., Coulson, A.R., Barrell, B.G., Smith, A.J.H. and Roe, B.A. (1980) Cloning in single-stranded bacteriophage as an aid to rapid DNA sequencing. J. Mol. Biol. 143: 161-178.

Tu, J. and Zillig, W. (1982) Organization of rRNA structural genes in the archaebacterium *Thermoplasma acidophilum*. Nucleic Acids Res. 10: 7231-7245.

Woese, C.R. (1987) Bacterial evolution. Microbiol. Rev. 51: 221-271.

Zillig, W., Palm, P., Reiter, W.D., Gropp, F., Puehler, G. and Klenk, H.P. (1988) Comparative evaluation of gene expression in archaebacteria. Eur. J. Biochem. 173: 473-482.

H. Legends to figures

Figure 1: Autoradiogram of Southern hybridization of ^{32}P labelled rRNA to gel containing sucrose gradient fractions of HindIII digested genomic DNA. The numbers on the top indicate the fraction number from the top of the gradient. Bateriophage λ DNA HindIII fragments were used as molecular size markers.

Figure 2: Restriction maps of the recombinant plasmids pHC8 (a) and pHH10 (b). The boxes indicate the *H. marismortui* inserted DNA. The darkened areas indicate the coding regions of 16S and 23S rRNA. The enzymes used are: B-BamHI, Bg-Bgl II, C-ClaI, E-EcoRI, H-HindIII, K-KpnI, P-PstI, S-SalI, Sm-SmaI, V-EcoRV, X-XhoI.

Figure 3: Protection of 5' end labelled DNA fragments by rRNA primary transcripts derived from the 5' upstream sequences of the two operons.
a A schematic presentation of the protected fragments: In the HH10 operon a 1860 nucleotides AvaI-HindIII fragment was used and in HC8 a 1160 nucleotides AvaI-AvaI fragment was used. **b** An autoradiogram of the gels used in the experiments. MWM -Molecular weight markers. A and C-controls without DNA. B and D- SI protected fragments of the HH10 and HC8 operons respectively.

Figure 4: Protection of 3' end labelled DNA fragments by rRNA primary transcripts and processed intermediates derived from the 16S-23S intergenic region. **a** A schematic presentation of the protected fragments of the 630 nucleotides AvaI-AvaI fragments derived from the two operons. **b** An autoradiogram of the gels used in the experiments. The description of the lanes is as in Figure 3.

Figure 5: Protection of 5' end labelled DNA fragments by rRNA primary transcripts and processed intermediates derived from the 16S-23S intergenic region. **a** A schematic presentation of the protected fragments: In the HH10 operon a 1650 nucleotides EcoRI-SmaI fragment was used and in the HC8 operon a 620 nucleotides EcoRV-EcoRV fragment was used. **b** An autoradiogram of the gels used in the experiments. The description of the lanes is as in Figure 3.

Figure 6: Protection of 3' end labelled DNA fragments by rRNA primary transcripts derived from the 3' down stream region of the operon. **a** A schematic presentation of the protected fragments: In the HH10 operon a 1800 nucleotides EcoRI-EcoRI fragment was used and in the HC8 operon a 2300 nucleotides EcoRI-XhoI fragment was used. **b** An autoradiogram of the gels used in the experiments. The description of the lanes is as in Figure 3.

Figure 7: Nucleotide sequences of the 5' ends of the 16S rRNA genes of the HC8 and HH10 clusters and their 5' flanking regions. The putative promoter sequences are indicated by boxes. The positions in the coding regions of the 16S rRNA genes at which mismatches exist are indicated by (*).

Figure 8: A comparison of the putative promoters of the rRNA operons HC8 and HH10 clusters of *H. marismortui* and the putative promoters of the single rRNA operon of *H. cutirubrum.*

I. Tables and Figures

Table 1: Restriction fragments of pHH10 and pHC8 that hybridized to 5S rRNA and tRNA.

	pHH10			pHC8		
Probe	5S rRNA	tRNA		5S rRNA	tRNA	
Restriction enzyme						
EcoRI	9,000*	9,000*	5,600	1,800	1,800	9,600
XhoI+BamHI	3,000	3,000	8,200*	7,400*	7,400*	4,800
PstI	8,000*	8,000*	5,600	1,500	1,500	9,500*

* Denote fragments that are attached to the vector.

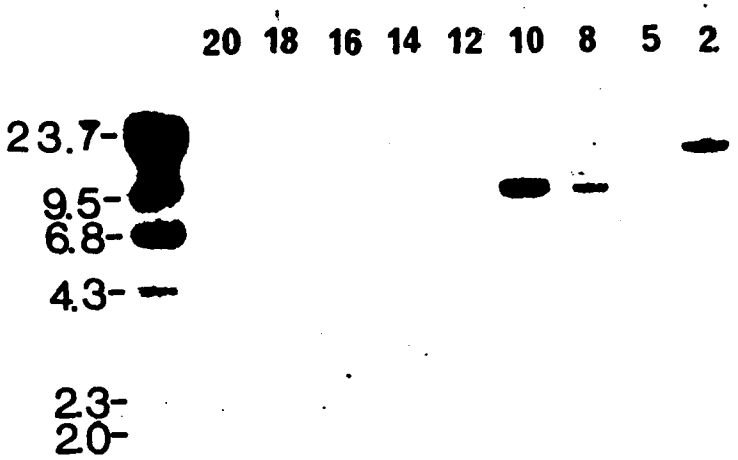

Figure 1

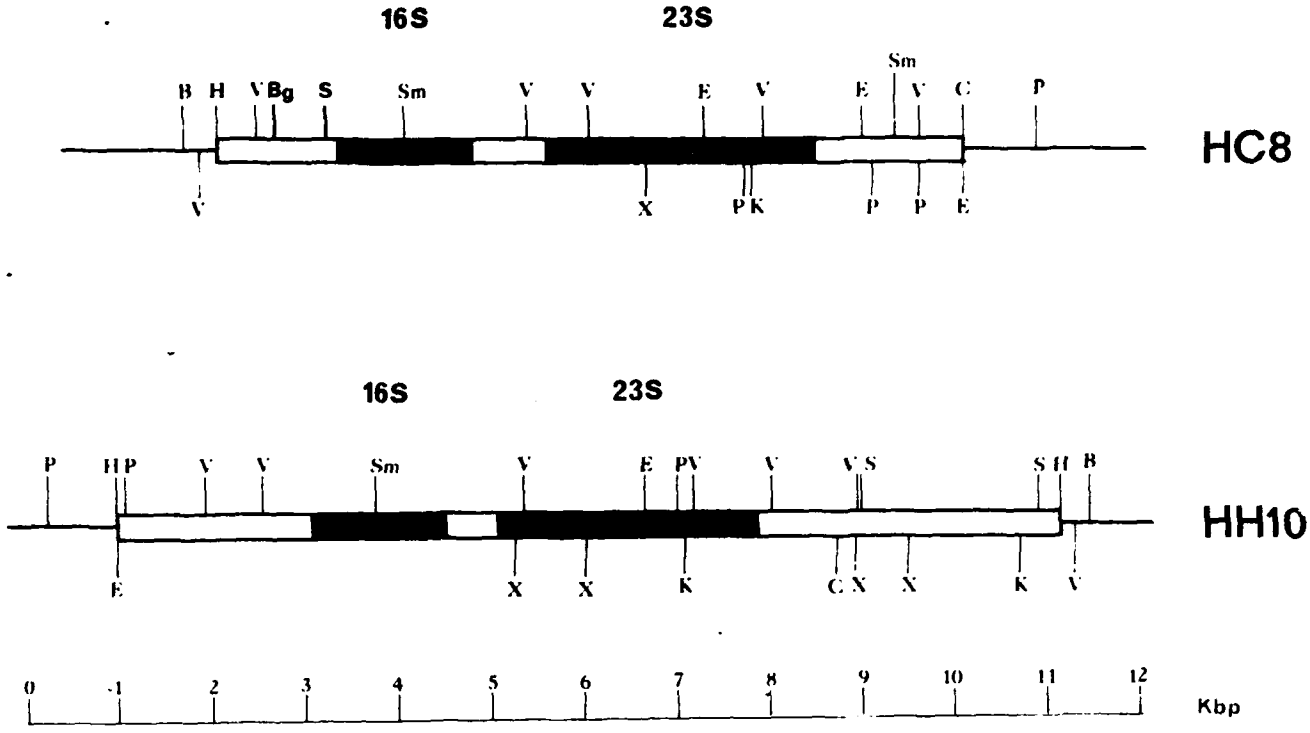
16S
23S
B H V Bg S Sm V V E V E Sm V C P
V X P K P P E
HC8
16S
23S
P H P V V Sm V E P V V V S S H B
E X X K C X X K V
HH10
0 1 2 3 4 5 6 7 8 9 10 11 12
Kbp

Figure 2

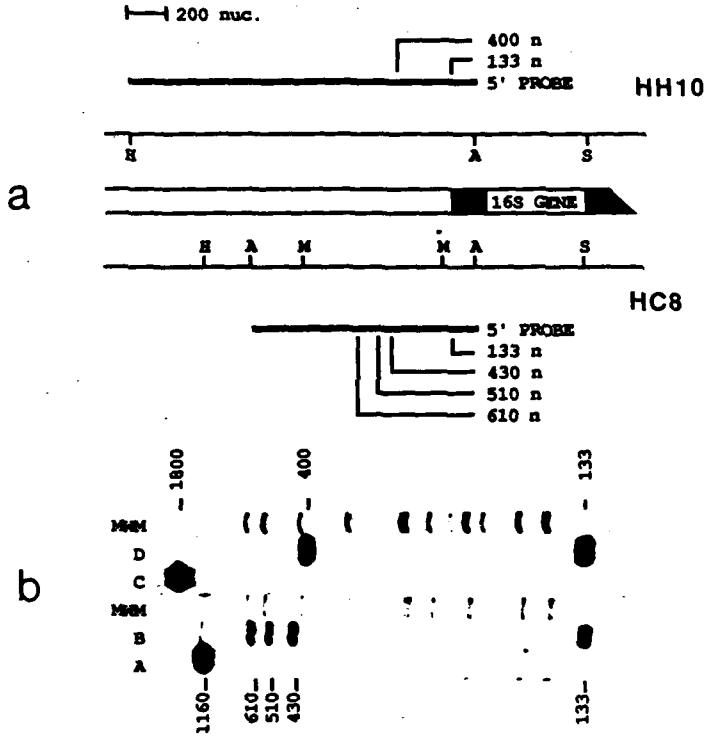
200 nuc.
400 n
133 n
5' PROBE
HH10
H
A
S
a
16S GENE
H A M M A S
HC8
5' PROBE
133 n
430 n
510 n
610 n
1800
400
133
MWM
D
C
MWM
B
A
1160
610
510
430
133
b

Figure 3

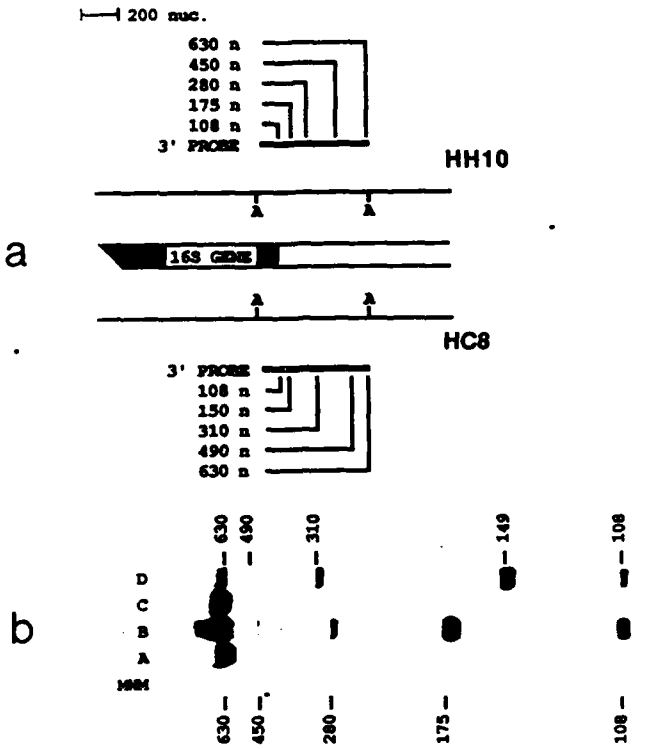
200 nuc.
630 n
450 n
280 n
175 n
108 n
3' PROBE
HH10
a
16S GENE
HC8
3' PROBE
108 n
150 n
310 n
490 n
630 n
b
D
C
B
A
MWM
630
490
310
149
108
630
450
280
175
108

Figure 4

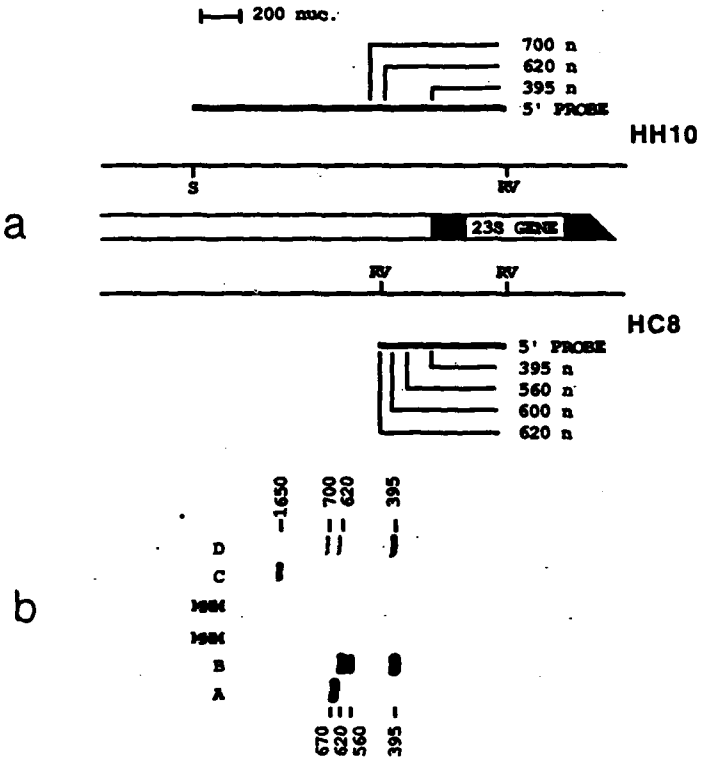
200 nuc.
700 n
620 n
395 n
5' PROBE
HH10
S
RV
a
23S GENE
RV
RV
HC8
5' PROBE
395 n
560 n
600 n
620 n
b
1650
700
620
395
D
C
B
A
670
620
560
395

Figure 5

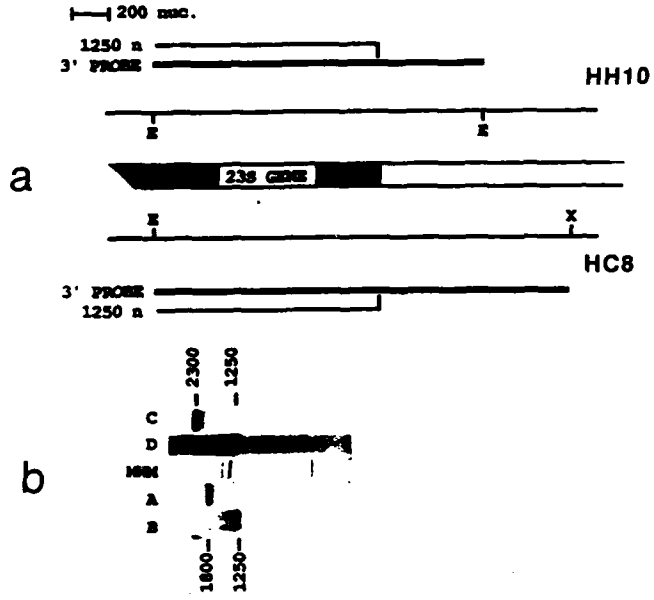
200 nuc.
1250 n
3' PROBE
HH10
E
E
a
23S GENE
E
X
HC8
3' PROBE
1250 n
2300
1250
C
D
A
B
1800
1250
b

Figure 6

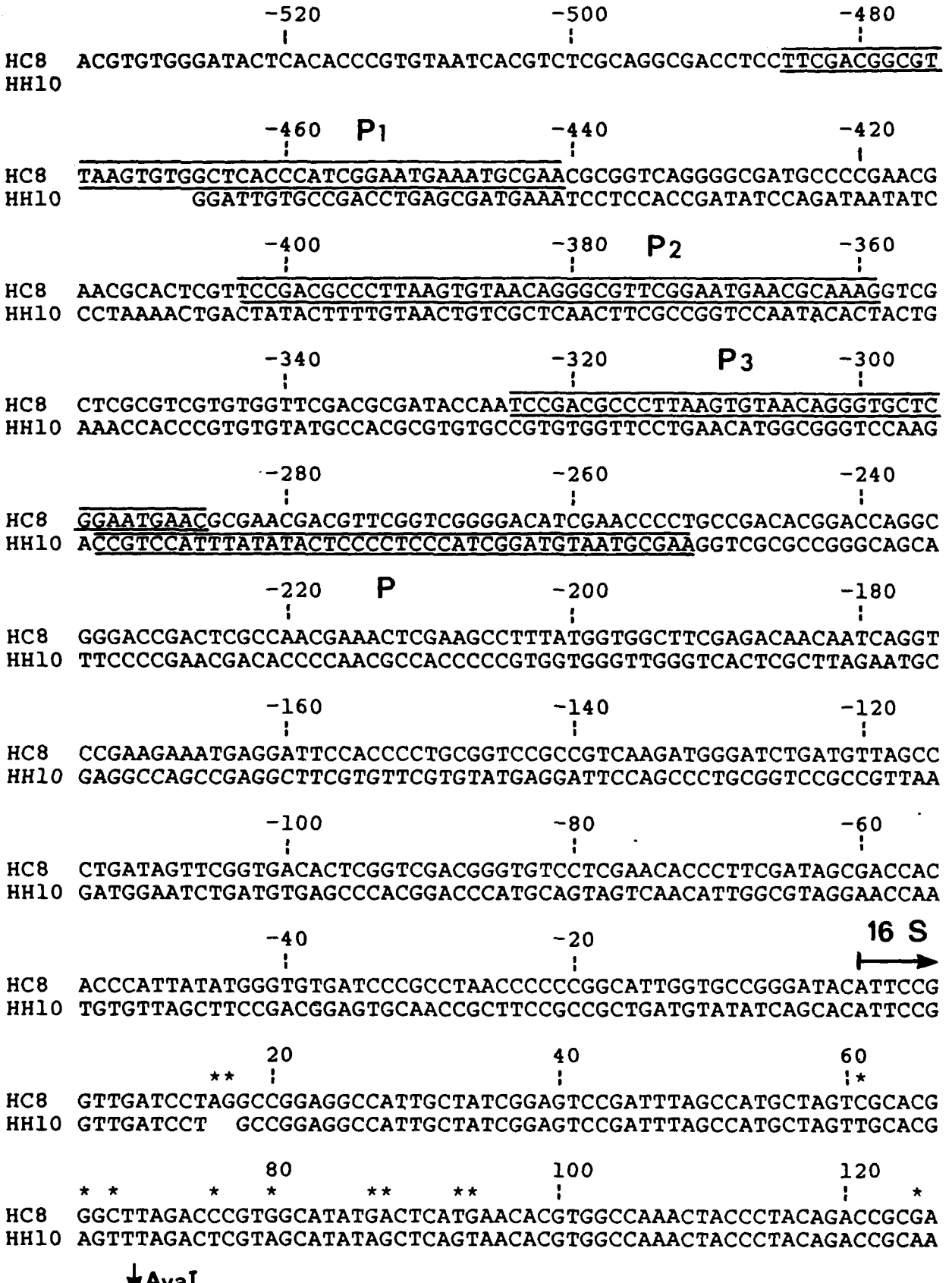
-520
-500
-480
HC8 ACGTGTGGGATACTCACACCCGTGTAATCACGTCTCGCAGGCGACCTCCTTCGACGGCGT
HH10
-460
P1
-440
-420
HC8 TAAGTGTGGCTCACCCATCGGAATGAAATGCGAACGCGGTCAGGGGCGATGCCCCGAACG
HH10 GGATTGTGCCGACCTGAGCGATGAAATCCTCCACCGATATCCAGATAATATC
-400
-380
P2
-360
HC8 AACGCACTCGTTCCGACGCCCTTAAGTGTAACAGGGCGTTCGGAATGAACGCAAAGGTCG
HH10 CCTAAAACTGACTATACTTTTGTAACTGTCGCTCAACTTCGCCGGTCCAATACACTACTG
-340
-320
P3
-300
HC8 CTCGCGTCGTGTGGTTCGACGCGATACCAATCCGACGCCCTTAAGTGTAACAGGGTGCTC
HH10 AAACCACCCGTGTGTATGCCACGCGTGTGCCGTGTGGTTCCTGAACATGGCGGGTCCAAG
-280
-260
-240
HC8 GGAATGAACGCGAACGACGTTCGGTCGGGGACATCGAACCCCTGCCGACACGGACCAGGC
HH10 ACCGTCCATTTATATACTCCCCTCCCATCGGATGTAATGCGAAGGTCGCGCCGGGCAGCA
-220
P
-200
-180
HC8 GGGACCGACTCGCCAACGAAACTCGAAGCCTTTATGGTGGCTTCGAGACAACAATCAGGT
HH10 TTCCCCGAACGACACCCCAACGCCACCCCCGTGGTGGGTTGGGTCACTCGCTTAGAATGC
-160
-140
-120
HC8 CCGAAGAAATGAGGATTCCACCCCTGCGGTCCGCCGTCAAGATGGGATCTGATGTTAGCC
HH10 GAGGCCAGCCGAGGCTTCGTGTTCGTGTATGAGGATTCCAGCCCTGCGGTCCGCCGTTAA
-100
-80
-60
HC8 CTGATAGTTCGGTGACACTCGGTCGACGGGTGTCCTCGAACACCCTTCGATAGCGACCAC
HH10 GATGGAATCTGATGTGAGCCCACGGACCCATGCAGTAGTCAACATTGGCGTAGGAACCAA
-40
-20
16 S
HC8 ACCCATTATATGGGTGTGATCCCGCCTAACCCCCCGGCATTGGTGCCGGGATACATTCCG
HH10 TGTGTTAGCTTCCGACGGAGTGCAACCGCTTCCGCCGCTGATGTATATCAGCACATTCCG
20
40
60
HC8 GTTGATCCTAGGCCGGAGGCCATTGCTATCGGAGTCCGATTTAGCCATGCTAGTCGCACG
HH10 GTTGATCCT GCCGGAGGCCATTGCTATCGGAGTCCGATTTAGCCATGCTAGTTGCACG
80
100
120
HC8 GGCTTAGACCCGTGGCATATGACTCATGAACACGTGGCCAAACTACCCTACAGACCGCGA
HH10 AGTTTAGACTCGTAGCATATAGCTCAGTAACACGTGGCCAAACTACCCTACAGACCGCAA
AvaI
HC8 TAACC
HH10 TAACC

Figure 7

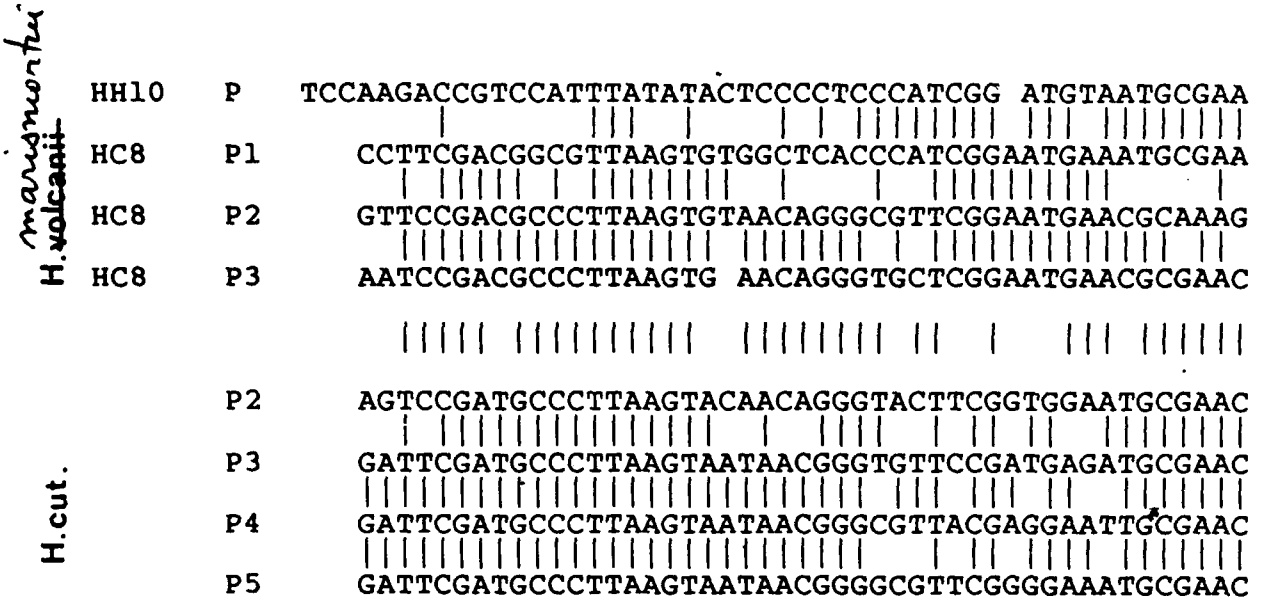
H. maasmanthii
H. volcanii
HH10 P TCCAAGACCGTCCATTTATATACTCCCCTCCCATCGG ATGTAATGCGAA
HC8 P1 CCTTCGACGGCGTTAAGTGTGGCTCACCCATCGGAATGAAATGCGAA
HC8 P2 GTTCCGACGCCCTTAAGTGTAACAGGGCGTTCGGAATGAACGCAAAG
HC8 P3 AATCCGACGCCCTTAAGTG AACAGGGTGCTCGGAATGAACGCGAAC
H. cut.
P2 AGTCCGATGCCCTTAAGTACAACAGGGTACTTCGGTGGAATGCGAAC
P3 GATTCGATGCCCTTAAGTAATAACGGGTGTTCCGATGAGATGCGAAC
P4 GATTCGATGCCCTTAAGTAATAACGGGCGTTACGAGGAATTGCGAAC
P5 GATTCGATGCCCTTAAGTAATAACGGGGCGTTCGGGGAAATGCGAAC

Figure 8

3. Conclusions and plans.

To determine whether the two promoter systems are transcribed at different stages in the cell cycle we are isolating appropriate RNA transcripts for hybridization with the individual clones. It should be possible to study the physiological significance of the two operons by determination of the extent of transcription at different growth rates. We will also attempt the fine analysis of the promoter region using a suitable reporter gene fused to the promoter regions.

We have not been able to establish a successful DNaseI footprint analysis at extreme salt concentration, nor were filter binding experiments and the use of chemically generated ·hydroxyl radical in solution to cut uncomplexed DNA backbones (cf. annual report 10/87) successful. We have now established that adequate practically identical nucleotide ladders can be obtained in low and high salt by methylation and depurination according to the Maxam-Gilbert sequencing procedure. We hope that this approach will allow footprint analysis with RNA polymerase or its components complexed to the sequenced DNA 5' upstream fragments and will lead to the isolation of transcription factors.

We hope in the next year to accomplish the specific polymerase binding assay to be used as a probe in the purification of the RNA polymerase and its transcriptional factors. Our final goal will then be to use these purified factors to reconstitute a promoter-dependent transcriptional assay.

A basic unsolved problem in the extreme halophilic systems is the mechanism of DNA protein interactions at KCl concentrations in the multimolar (4M) range, the control of transcription and replication under these conditions, and the organization of the halobacterial chromosome in contrast to the E.coli chromosome. Unfortunately not much of major significance is known about the latter, when compared to our knowledge of eukaryotic systems. We are planning to isolate nucleic acid binding proteins in halobacterial systems and to study their mutual interactions, to advance our knowledge on the halobacterial chromosome organization and protein nucleic acid interactions in general and specifically in transcription.

4. Personnel and general comments.

Yohevet Lamed M.Sc. left the project on November 1, 1987 for personal reasons and was replaced on November 4, 1987 by Tova Shimon, M.Sc. Emanuel Yakobson, Ph.D. left the project on December 7, 1987. Professor Patrick P. Dennis, of the University of British Columbia, Vancouver, Erna and Jacob Michael Visiting Professor at the Weizmann Institute, stayed in our laboratory between May 1 and August 11, 1987. Successful collaboration between our two groups is in progress. On June 15, 1988 we were joined by Tom Yager, Ph.D., from the University of Oregon, who will stay for two years in our laboratory to study protein nucleic acid interaction in halophilic as compared to nonhalophilic systems.

Results of our work to-date were presented at the meeting on "Archaebacteria: Genome structure, transcription, translation and gene

expression", Victoria, B.C., July 31 - August 5, 1988. Publication of work accomplished so far is in progress. We are organizing the 12th Rothschild School on Molecular Biophysics on "Modern Aspects of Halophilism", March 26 to April 6, 1989, Rehovot, Israel, in which evolutionary, genetic, structural and energetic aspects will be presented.